

FIG. 1

1	5	10
ATG GCT CCA ATG ACT CAG ACT ACT TCT CTT AAG ACT TCT		
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser		
15	20	25
TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA		
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr		
30	35	
CAC TTA AAG CAG CCA CCT TTG CCT TTG CTG GAC TTC AAC		
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn		
40	45	50
AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT		
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn		
55	60	
AAC CTT CGA AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT		
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala		
65	70	75
GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC ATT		
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile		
80	85	90
CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC		
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala		
95	100	
GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC		
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp		
105	110	115
TGG AAT GAA TTC CGT CGT AAA CTG ACC TTC TAT CTG AAA		
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys		
120	125	
ACC TTG GAG AAC GCG CAG GCT CAA CAG ACC ACT CTG TCG		
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser		
130		
CTA GCG ATC TTT TAA TAA	(SEQ ID NO: 144)	
Leu Ala Ile Phe END END	(SEQ ID NO: 138)	

C
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aa20 1 ATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTGCTGGACTTCAACAAC
-----+-----+-----+-----+-----+-----+ 60
IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn -

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61 CTCAATGGTGAAGACCAAGATATCCTGATGGAAATAACCTTCGTCTGTCCTCAAACCTCGAG
-----+-----+-----+-----+-----+-----+ 120
LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu -

P N
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121 GCATTCAACCGTGTGTCAAGTCTCTGCAGAAATGCAT [SEQ ID NO:145]aa70
-----+-----+-----+-----+-----+ 157
AlaPheAsnArgAlaValLysSerLeuGlnAsnAla [SEQ ID NO:146]

Clal to Nsil Replacement Fragment

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1 CCATGGCTCCAATGACTCAGACTACTTCTCTTAAGACTTCTTGGGTAACTGCTCTAACA
-----+-----+-----+-----+-----+-----+ 60
GGTACCGAGGTTACTGAGTCTGATGAAGAGAATTCTGAAGAACCCAATTGACGAGATTGT

MetAlaProMetThrGlnThrThrSerLeuLysThrSerTrpValAsnCysSerAsnMet

C
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61 TGATCGATGAAATTATAACACACTTAAAGCAGCCACCTTTGCCTTTGCTGGACTTCAACA
-----+-----+-----+-----+-----+-----+ 120
ACTAGCTACTTTAATATTGTGTGAATTTTCGTCGGTGAAACGGAAACGACCTGAAGTTGT

IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn

121 ACCTCAATGGGGAAGACCAAGACATTCTGATGGAAAATAACCTTCGAAGGCCAAACCTGG
-----+-----+-----+-----+-----+-----+ 180
TGGAGTTACCCCTTCTGGTTCTGTAAGACTACCTTTTATTGGAAGCTTCCGTTTGGACC

LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu

N
S
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I

181 AGGCATTCAACAGGGCTGTCAAGAGTTTACAGAATGCATCAGCAATTGAGAGCATTCTTA
-----+-----+-----+-----+-----+-----+ 240
TCCGTAAGTTGTCCCGACAGTTCTCAAATGTCTTACGTAGTCGTAACTCTCGTAAGAAT

AlaPheAsnArgAlaValLysSerLeuGlnAsnAlaSerAlaIleGluSerIleLeuLys

240 AAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATA
-----+-----+-----+-----+-----+-----+ 300
TTTtagAGGACGGTACAGACGGGGACCGGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTAT

AsnLeuLeuProCysLeuProLeuAlaThrAlaAlaProThrArgHisProIleHisIle

E
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301 TCAAGGACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGG
 -----+-----+-----+-----+-----+-----+ 360
 AGTTCCTGCCACTGACCTTACTTAAGGCAGCATTGACTGGAAGATAGACTTTTGGAAAC

LysAspGlyAspTrpAsnGluPheArgArgLysLeuThrPheTyrLeuLysThrLeuGlu

N
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n
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I

361 AGAACGCGCAGGCTCAACAGACCACTCTGTCGCTAGCGATCTTTTAATAAGCTT
 -----+-----+-----+-----+-----+ 414
 TCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATCGCTAGAAAATTATTCGAA

AsnAlaGlnAlaGlnGlnThrThrLeuSerLeuAlaIlePheEndEnd

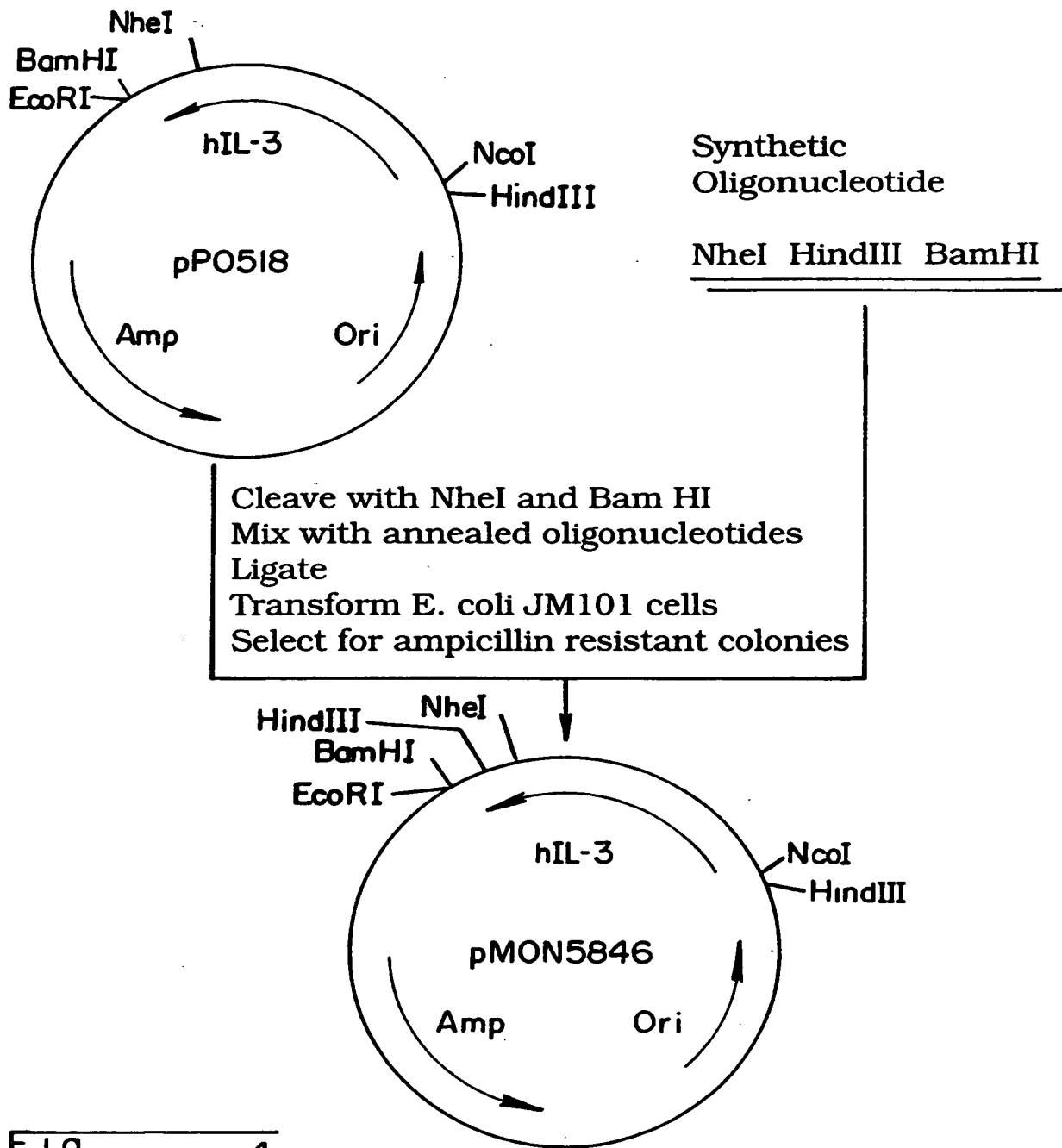
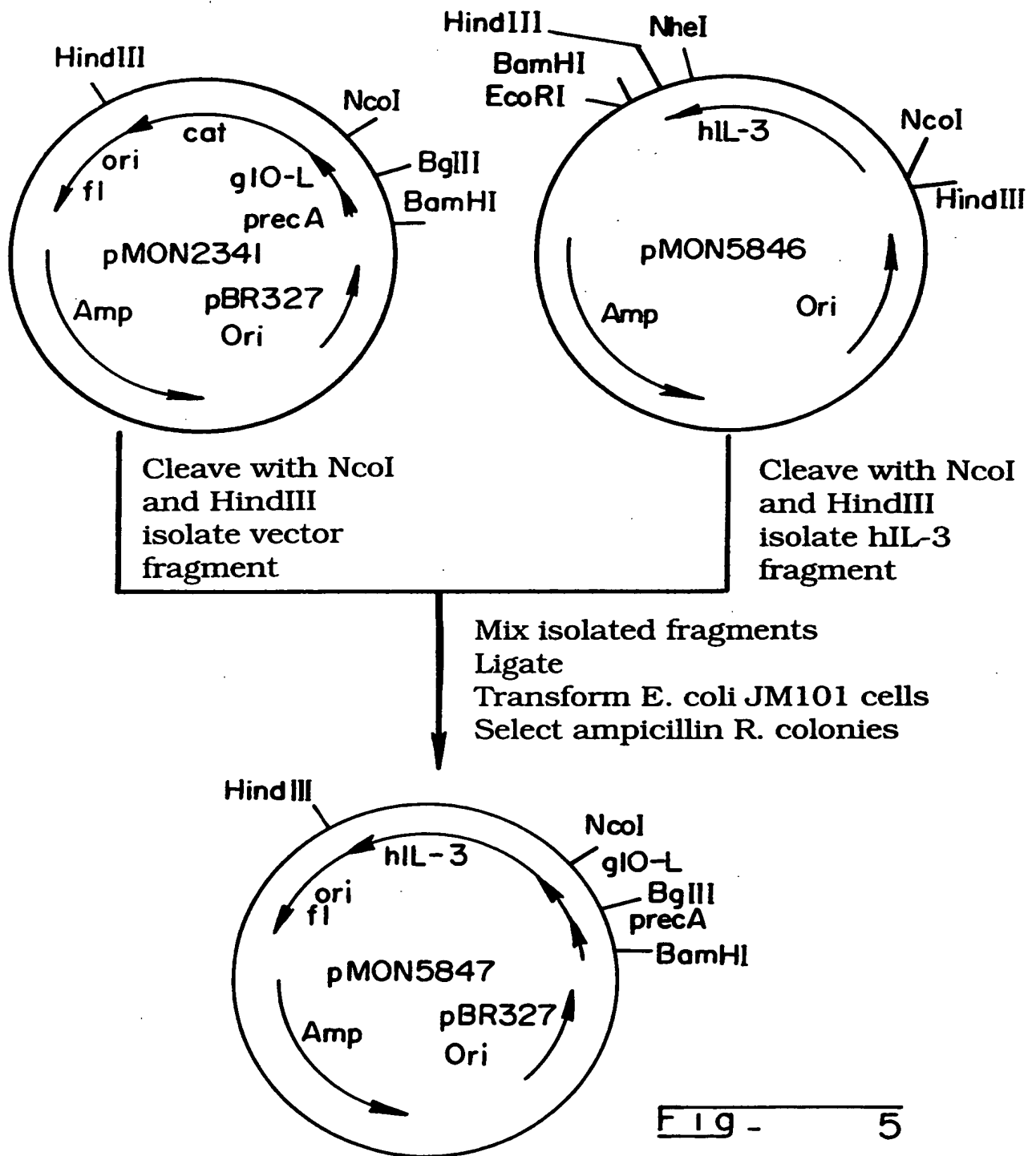
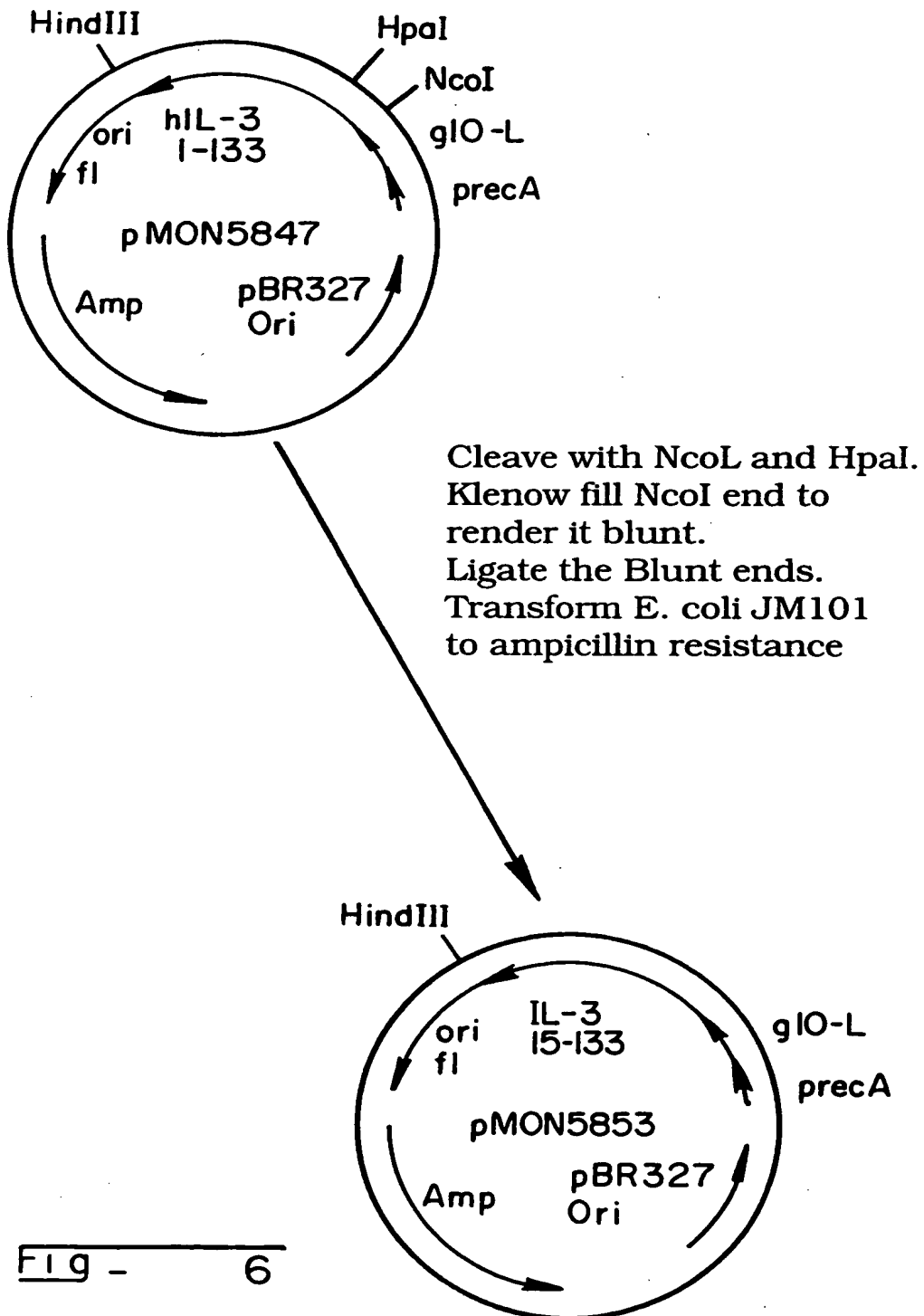
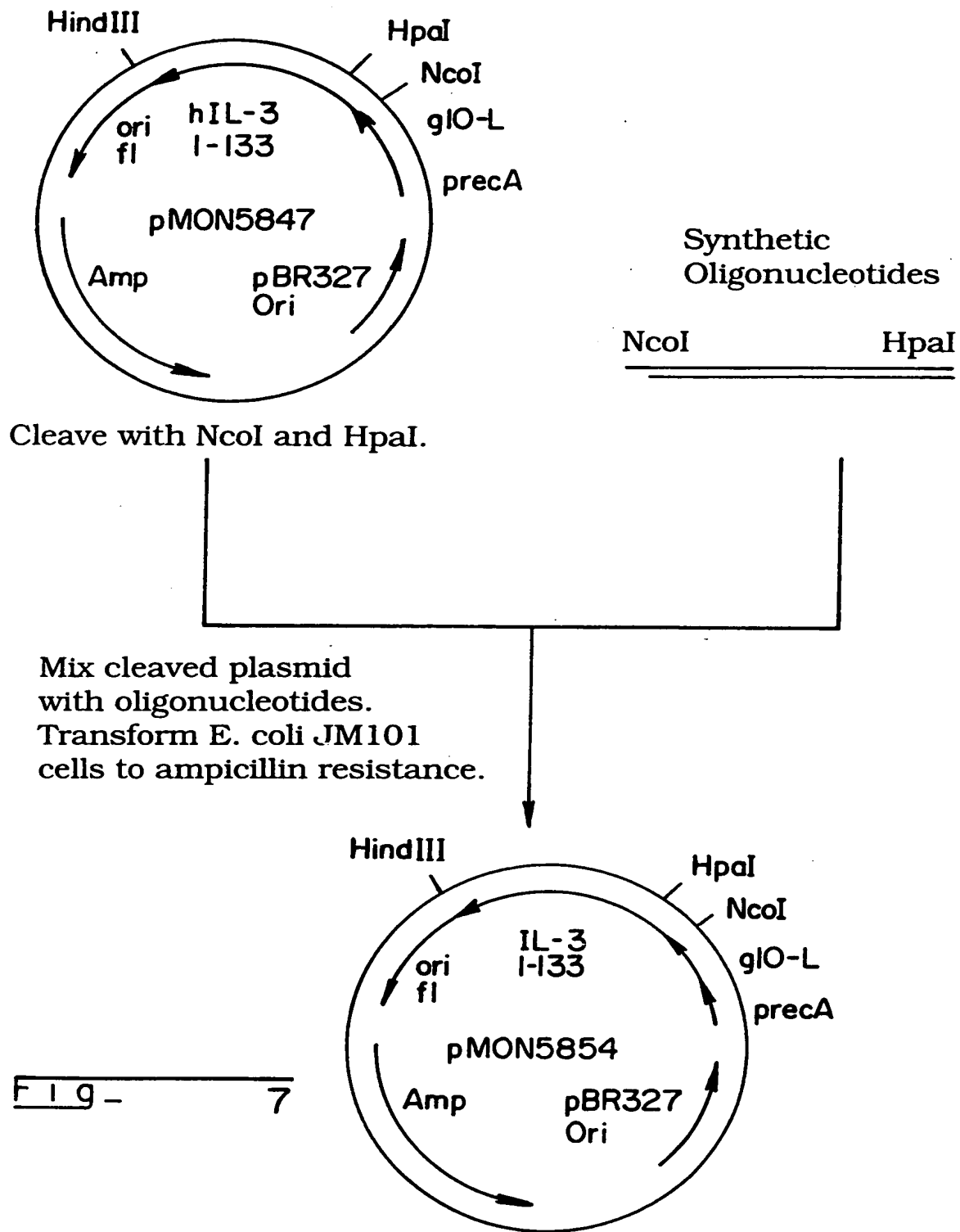


Fig- 4







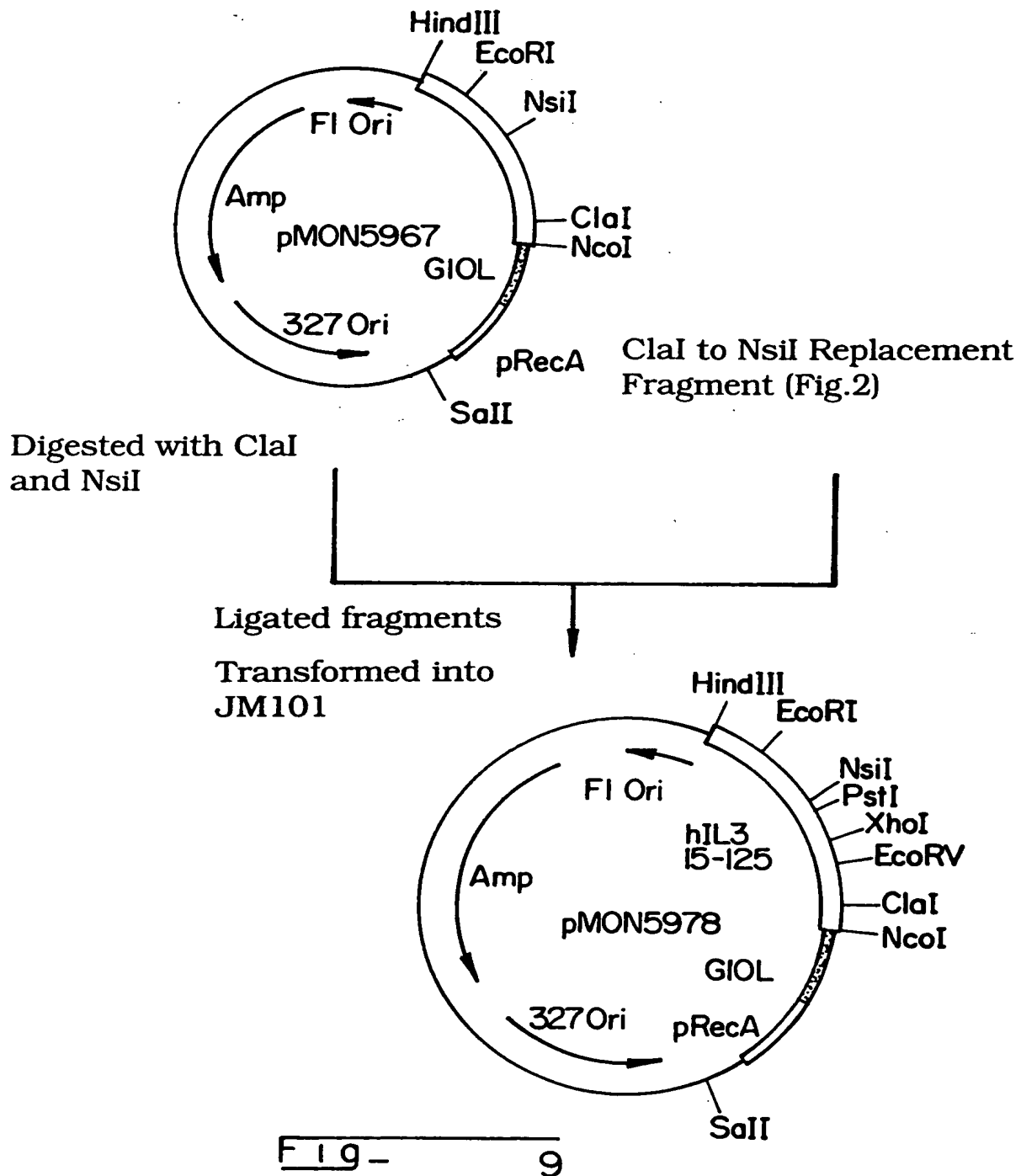
1 ATGATGATTACTCTGCGCAAACTTCTCTGGCGGTTGCCGTGCAGCGGGCGTAATGTCT 60
TACTACTAATGAGACGCGTTTGAAGGAGACCGCCAACGGCAGCGTCGCCCGCATACAGA

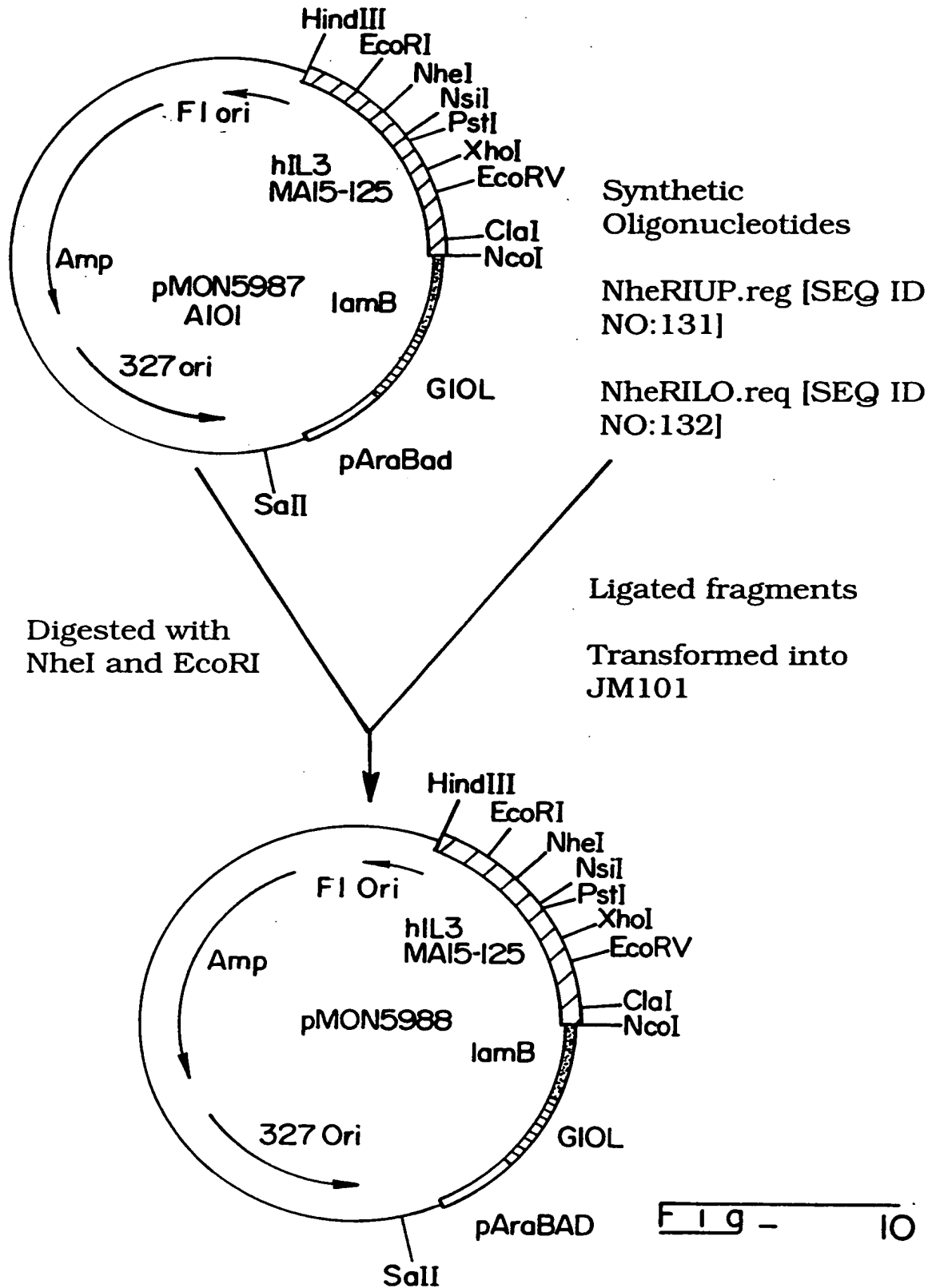
MetMetIleThrLeuArgLysLeuProLeuAlaValAlaAlaGlyValMetSer

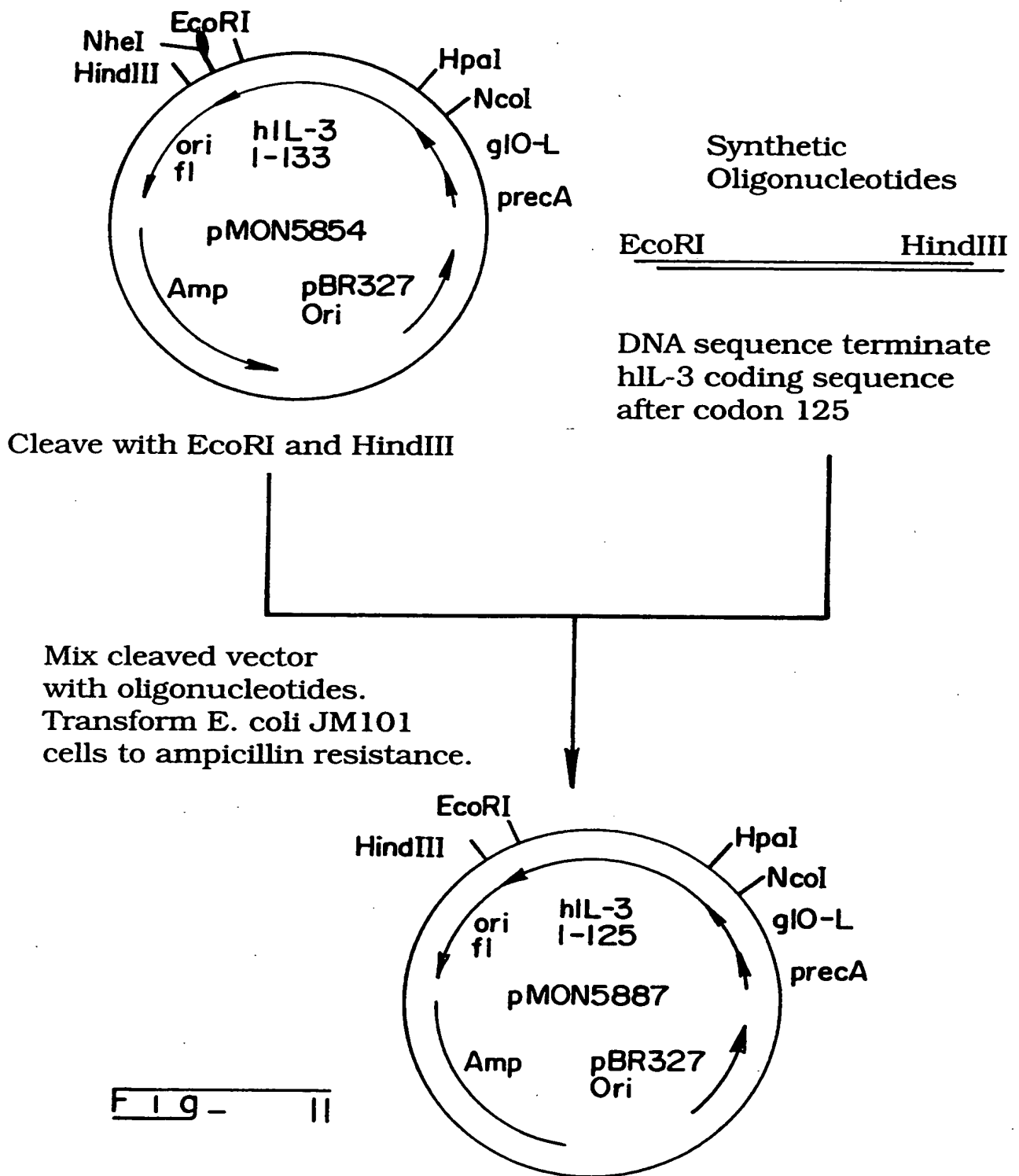
N
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61 GCTCAGGCCATGGCTAACTGC [SEQ ID NO: 149]
CGAGTCCGGTACCGATTGACG [SEQ ID NO: 150]
AlaGlnAlaMetAlaAsnCys [SEQ ID NO: 14]

lamB Signal Peptide







5' CATGGCTAACTGCTCTAACATGAT 3'
SEQ ID NO:151

3' CGATTGACGAGATTGTACTAGC 5'
SEQ ID NO:152

Annealed
Oligonucleotides

Ligated fragments

Transformed into
JM101

Digested with NcoI
and ClaI

Gel purified 4263 bp
fragment

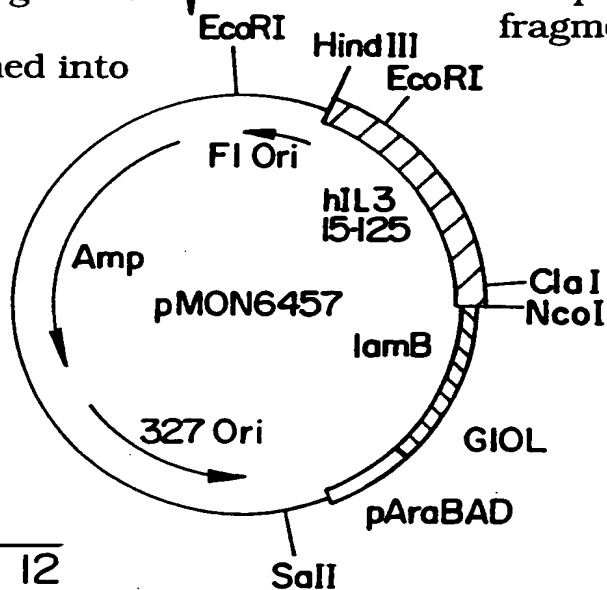
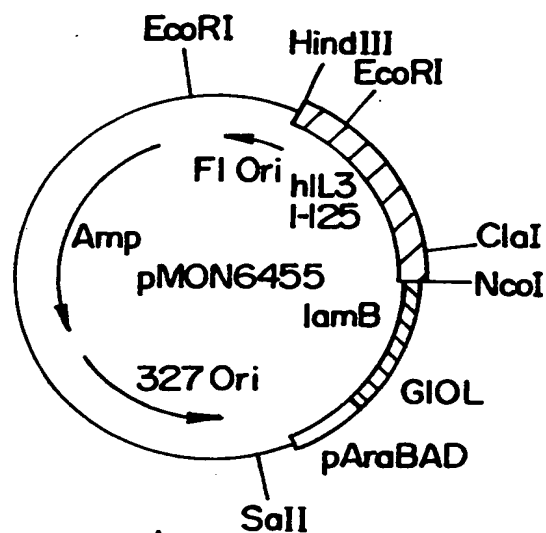
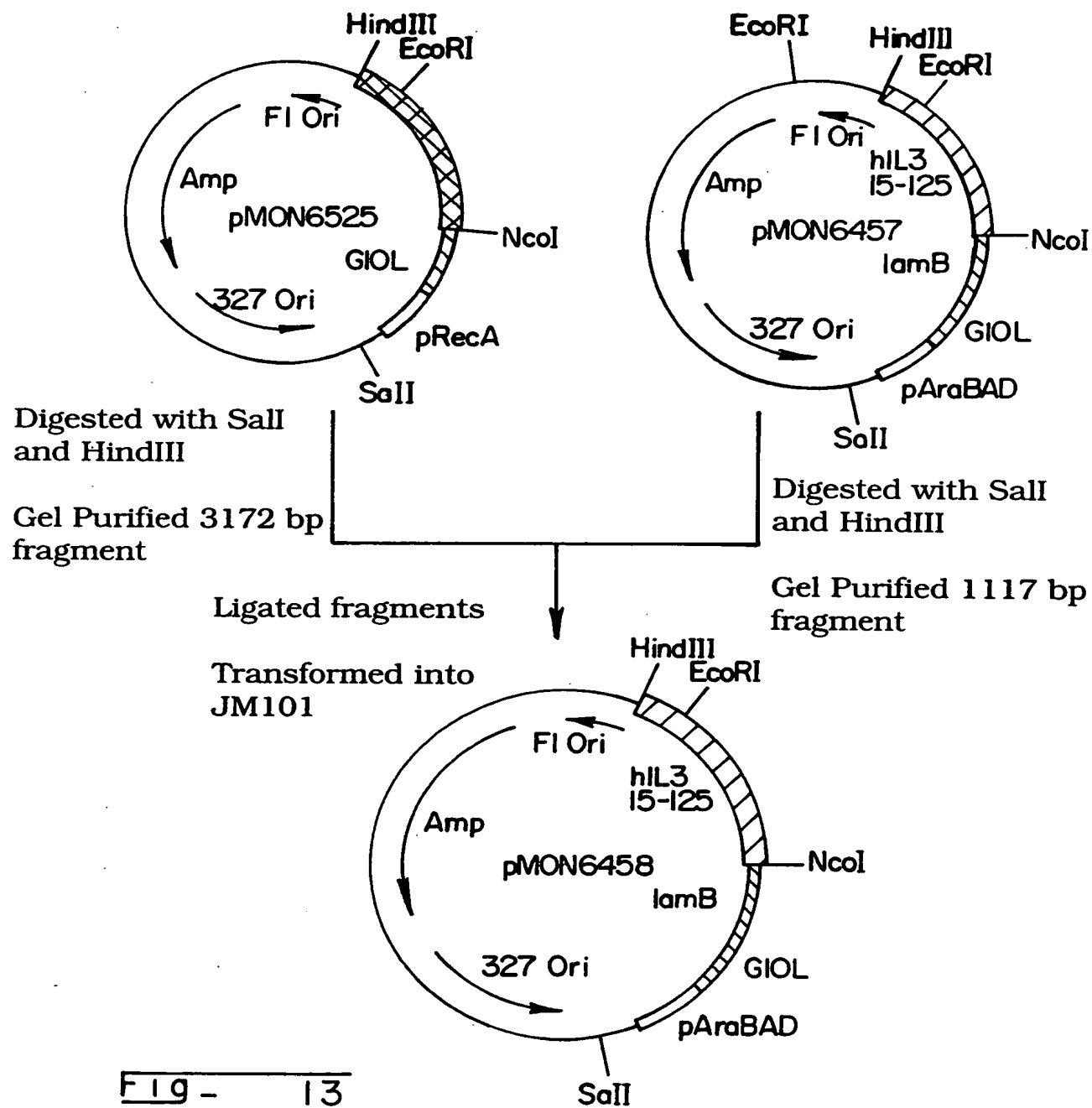


Fig - 12



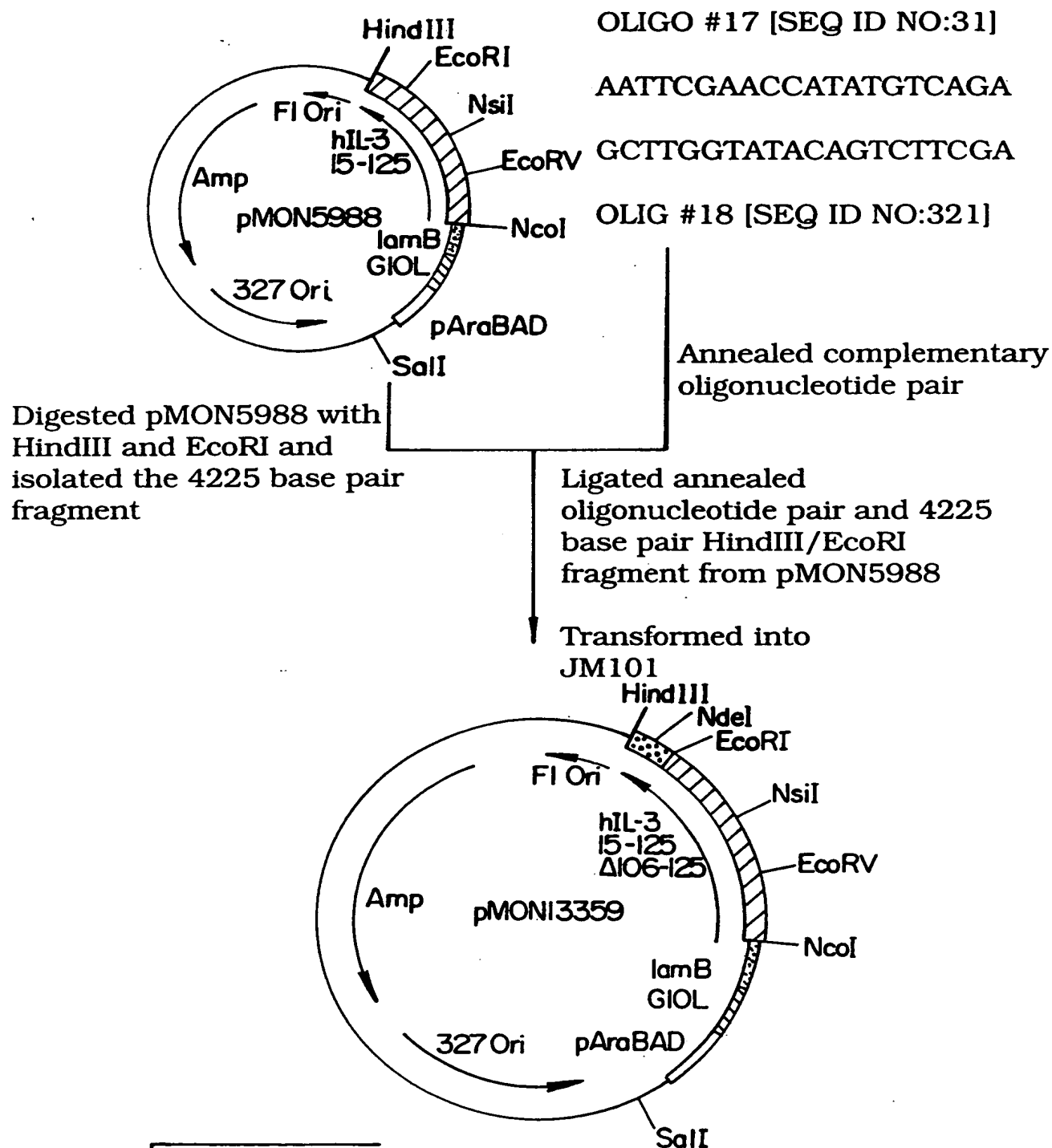
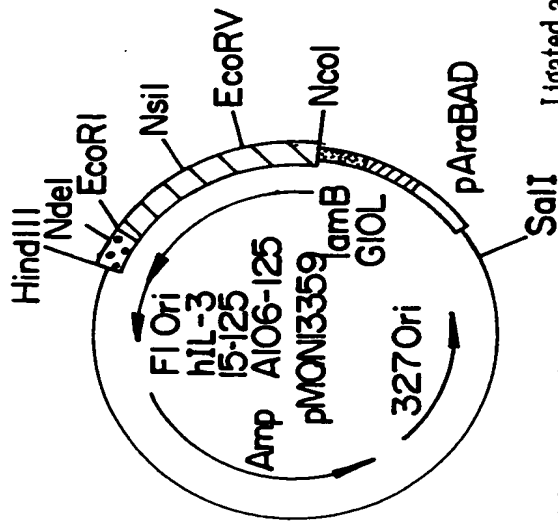


FIG - 14



Digested pMON13359 with HindIII and EcoRI and isolated the 4225 base pair fragment

Ligated annealed oligonucleotide pairs and 4225 base pair HindIII/EcoRI fragment from pMON13359

Transformed into JM101

Annealed complementary oligonucleotide pairs

OLIGO #45 [SEQ ID NO:59]
 5' AATTCGGGAAACTGACGTTCTATCTGGTT 3'
 3' GGCCCTTTTGACTGCAAGATAGACCAAAGGGAAC TCG 5'
 OLIGO #46 [SEQ ID NO:60]

OLIGO #49 [SEQ ID NO:63]
 5' TCCCTTGAGCAAGCGCAGGAACACAGTAATA 3'
 3' TTCGCGTCCTTGTTGTCATTATTCGA 5'
 OLIGO #50 [SEQ ID NO:64]

Fig - 15

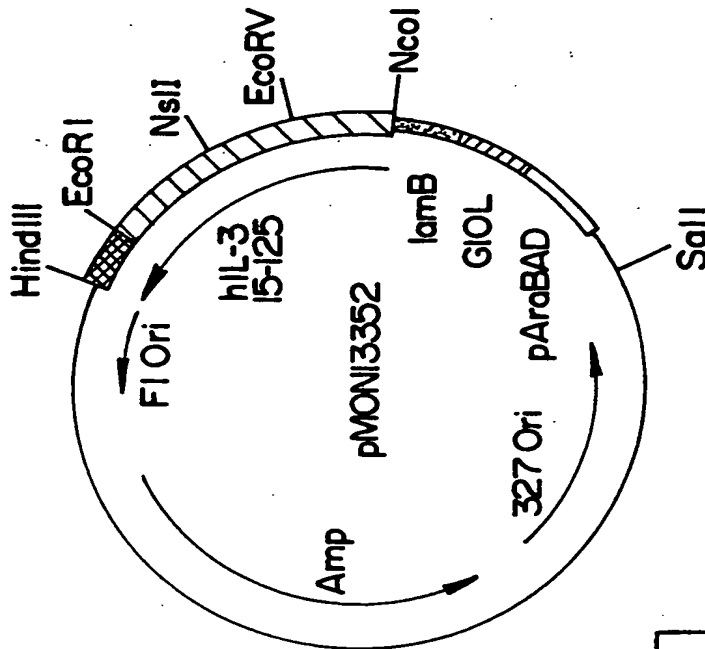
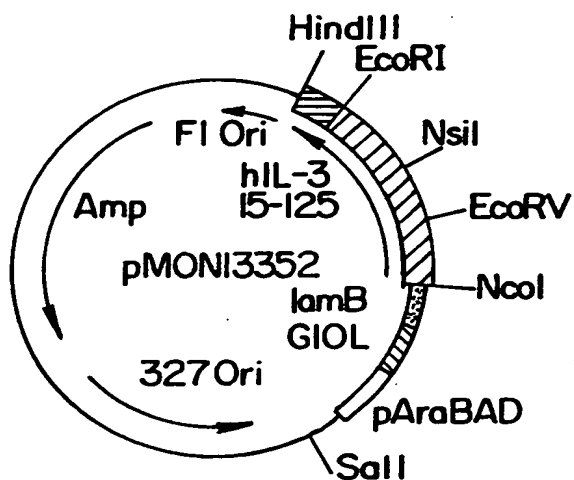
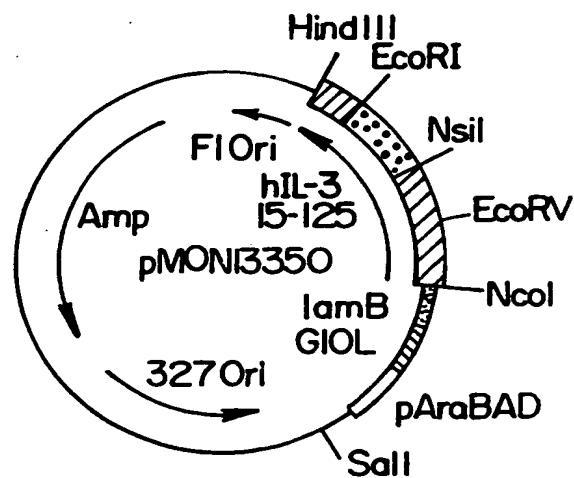


Fig - 16



Digested pMONI3352 with Nsil and EcoRI and isolated the 4178 base pair fragment



Digested pMONI3350 with Nsil and EcoRI and isolated the 111 base pair fragment

Ligated fragments

Transformed into JM101

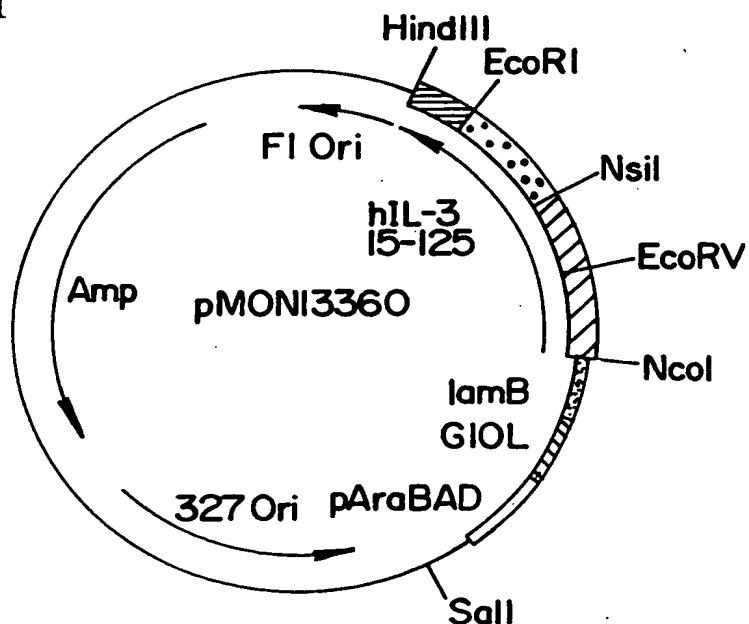
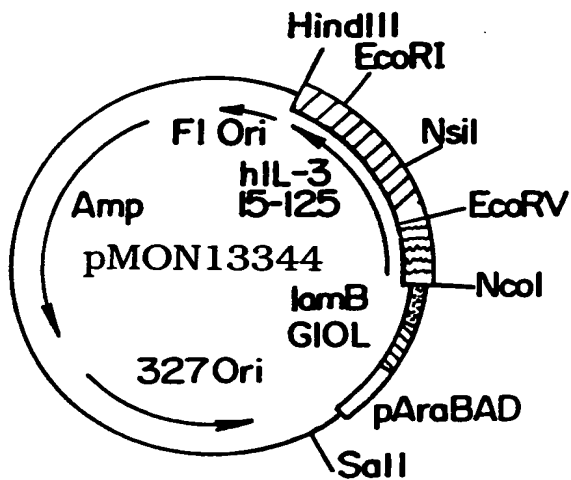
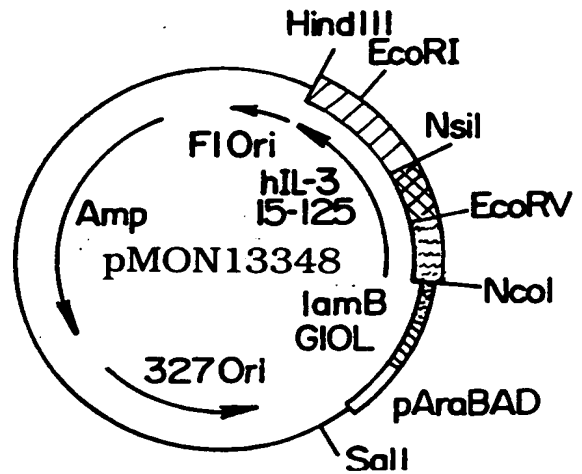


Fig - 17



Digested pMON13344 with Nsil and EcoRV and isolated the 4218 base pair fragment



Digested pMON13348 with Nsil and EcoRV and isolated the 71 base pair fragment

Ligated fragments

Transformed into JM101

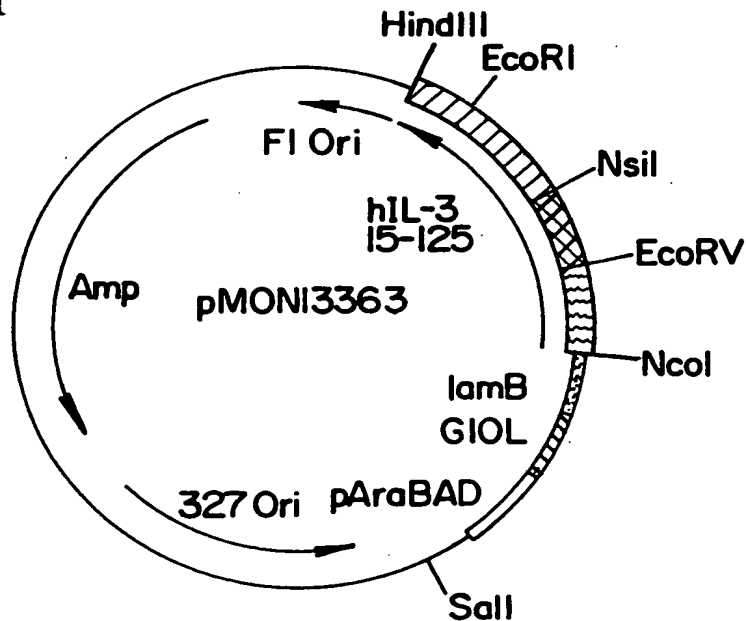
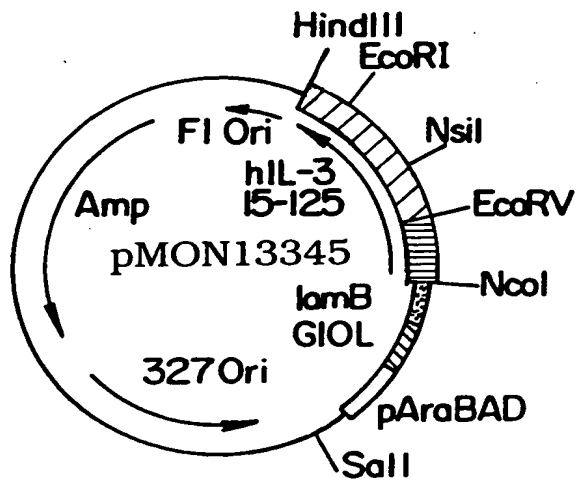
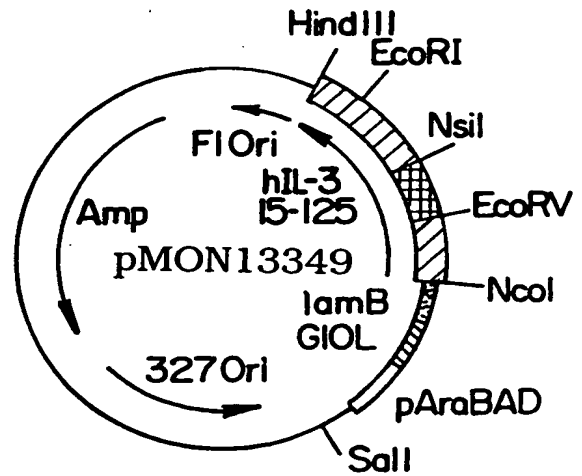


Fig - 18



Digested pMON13345 with
NsiI and EcoRV and
isolated the 4218 base pair
fragment



Digested pMON13349 with
NsiI and EcoRV and
isolated the 71 base pair
fragment

Ligated fragments

Transformed into
JM101

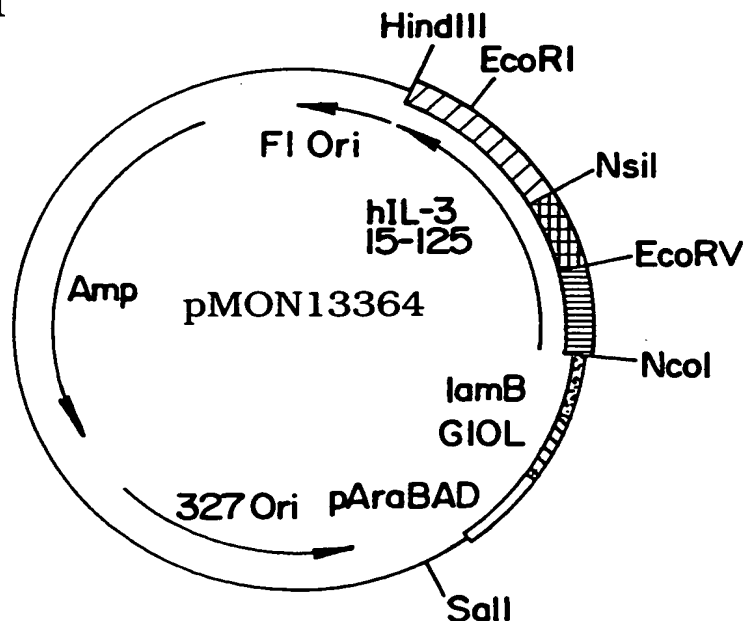
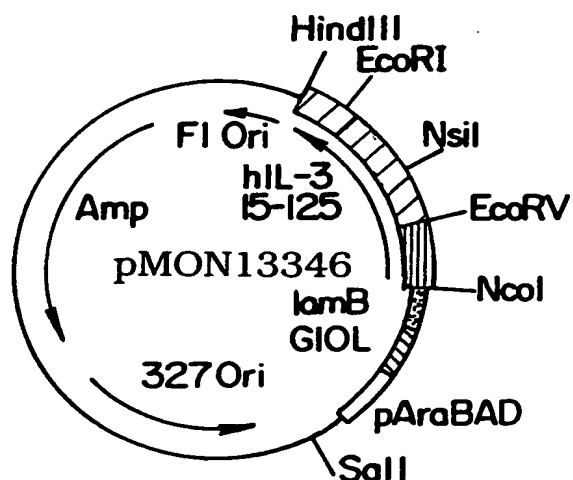
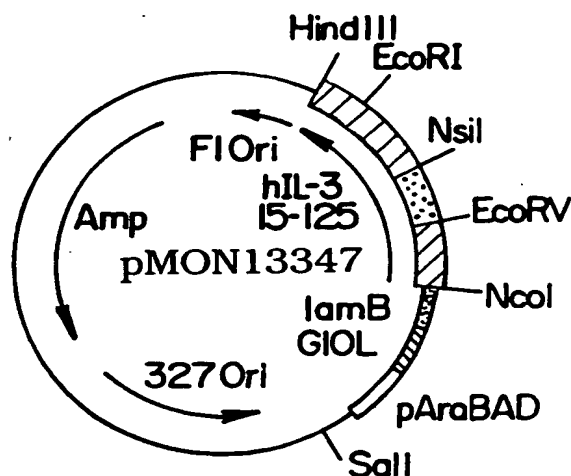


Fig - 19



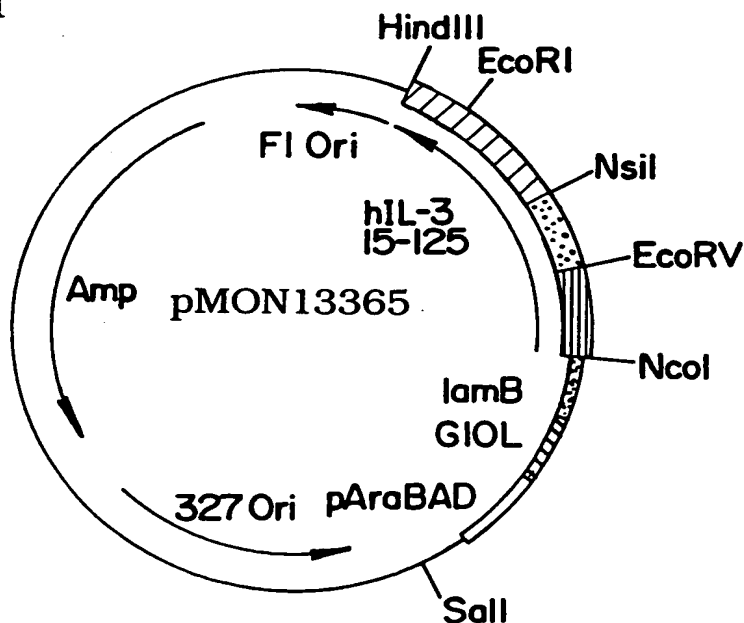
Digested pMON13346 with NsiI and EcoRV and isolated the 4218 base pair fragment

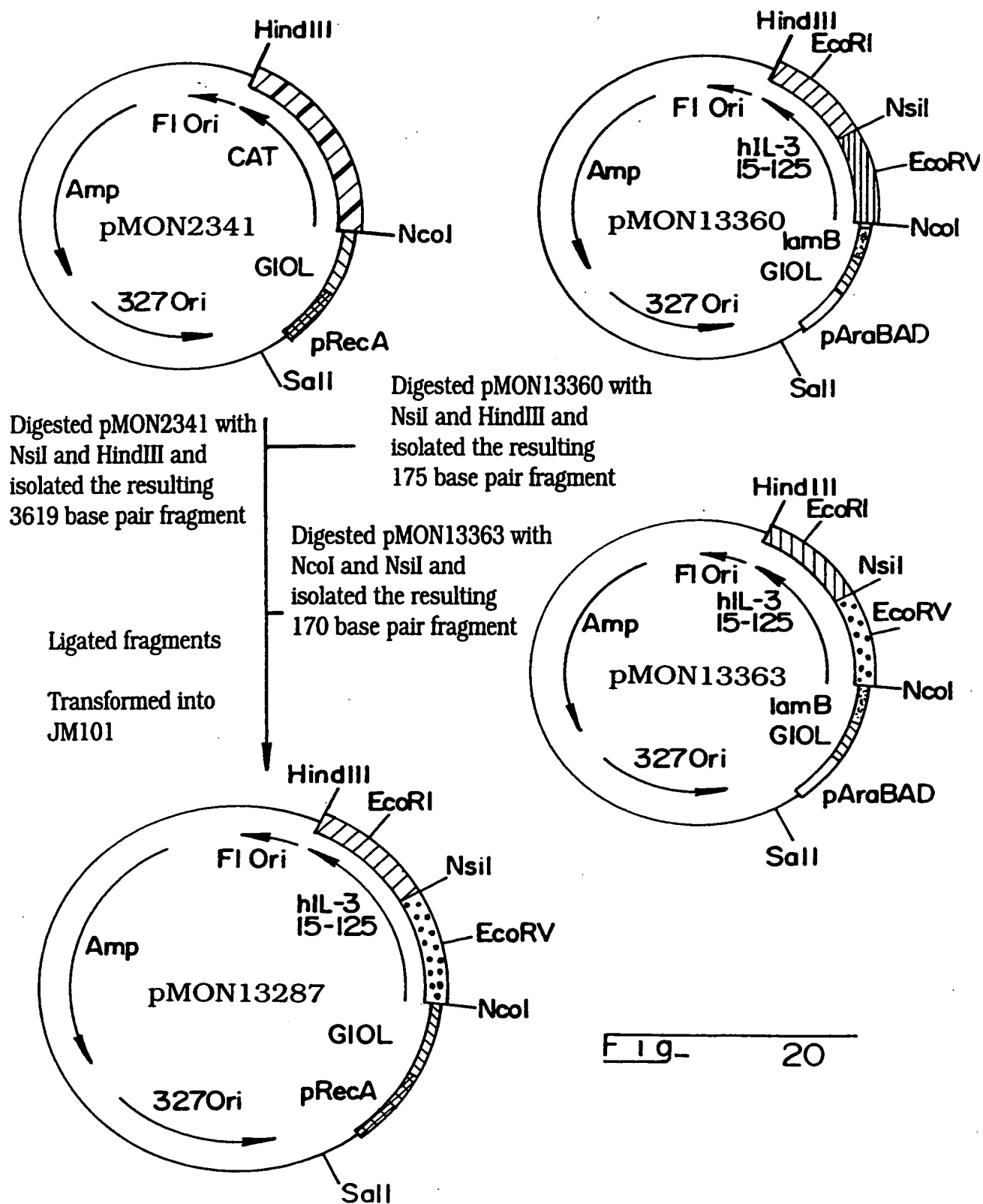


Digested pMON13347 with NsiI and EcoRV and isolated the 71 base pair fragment

Ligated fragments

Transformed into JM101





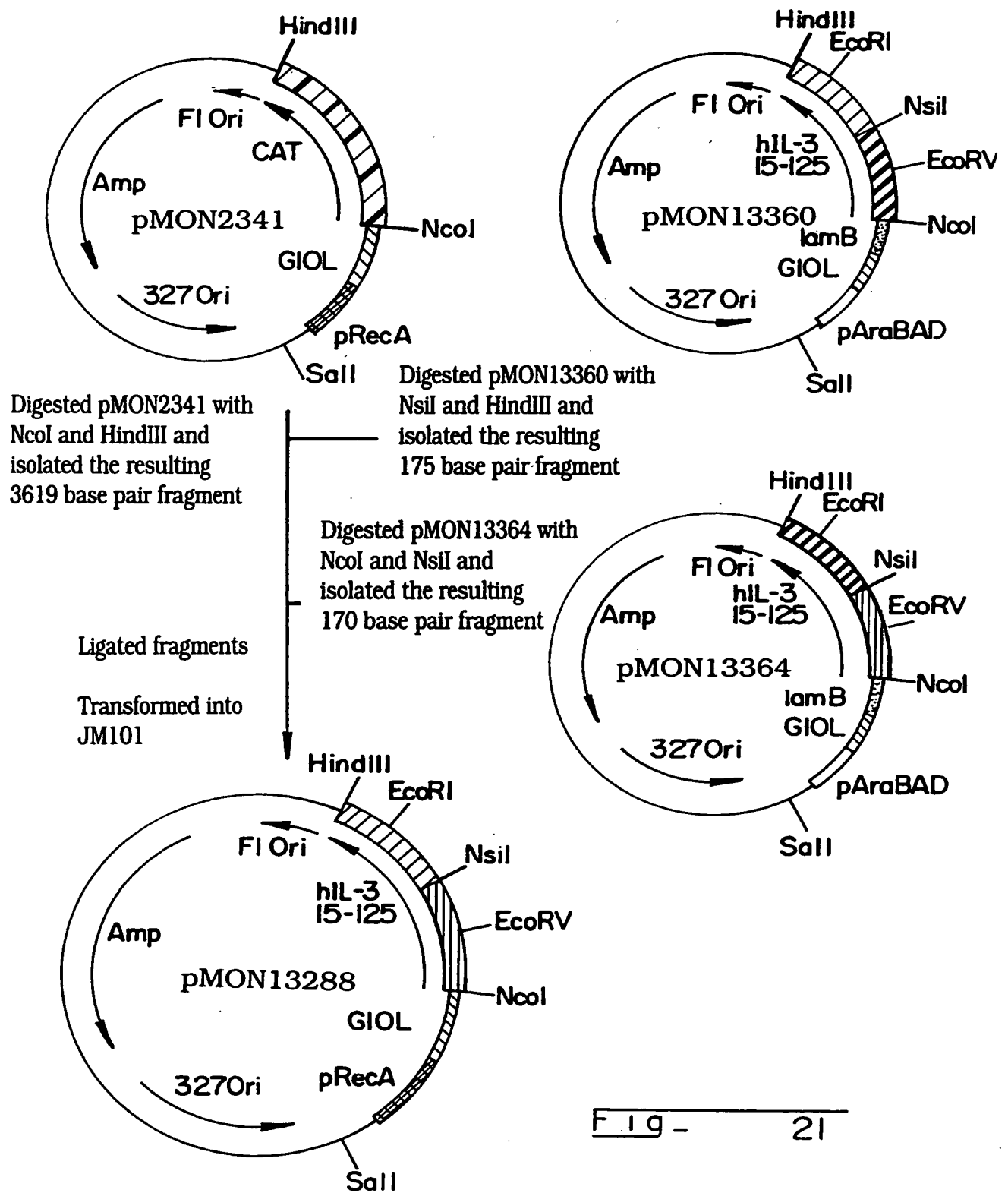


Fig - 21

